Immunotoxicology in Marine Invertebrates:

Effects of Manganese on Immune Response

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Examinator: Prof. Michael Thorndyke, Institutionen för Marin Ekologi, Göteborgs Universitet

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Abstract

Manganese, Mn, is an abundant element in nature, particularly in soft bottom sediments of the oceans and in bedrock. The metal is predominantly bound to the sediment in the colloid state, MnO_2. Eutrophication caused by the high nutrient load in coastal waters together with over-fishing cause cascade effects in the ecosystem increasing the algal blooms and enhancement of hypoxic condition over large bottom areas. During hypoxic events MnO_2 is reduced and released into the bottom water as bioavailable ions, Mn^{2+}. Mn is essential for several metabolic and enzymatic processes and is necessary for both animals and plants. Elevated levels though, are toxic and severe effects on the nervous system have been known for long. In addition, previous studies have shown an impaired immune system of the bottom living lobster, *Nephrops norvegicus*, when exposed to concentrations that are realistic to find in nature. In this study I aimed to investigate if immunotoxic effects of manganese are general also for other marine invertebrates.

It is widely accepted that invertebrates do not have a documented so called *adaptive* immune response. They lack the genes, proteins and cells for the highly specific recognition and the long-term memory as found in vertebrates. Invertebrates primarily rely on the *innate* immune system to effectively combat a wide array of microbial pathogens. The innate immune system comprises of a first line of defence systems such as coagulation and melanization reactions, often followed by cellular reactions such as phagocytosis, encapsulation and production of antimicrobial substances. Many innate immune reactions are highly evolutionary conserved and are found throughout the whole animal kingdom. In aquatic invertebrates the open coelom or semi-open haemal circulatory system continuously expose them to potential pathogens and their immune response has proved to be exceptionally efficient in pathogen elimination as witnesses by the invertebrates' evolutionary success.

In this thesis species from three different phyla within the Bilaterians were investigated; the Norway lobster, *Nephrops norvegicus* (Crustacea), the blue mussel *Mytilus edulis* (Mollusca) and the common sea star, *Asterias rubens* (Echinodermata), differing in preferred habitats, feeding behaviour and somewhat in their strategies of immune defence. Studies were made on molecular, cellular and organism levels. On molecular and cellular levels we investigated the effects of manganese on the renewal of haemocytes (proliferation and differentiation of new cells), manganese effects on viability of haemocytes and the stress responses measured in both haemocytes and haematopoietic tissue. On the whole organism we investigated the effect of manganese on the ability for the animals to clear their cavity form injected bacteria.

The results of this thesis show that Mn in concentrations found in bottom waters affects the immune system of marine invertebrates differently. In *N. norvegicus* the metal severely suppresses the number of circulating haemocytes by inducing apoptosis, programmed cell death. The impaired immunity made them more susceptible to infections, which was also found in *M. edulis*. In *A. rubens* the same Mn concentration seemed to have a stimulating effect (hormesis) on the haematopoiesis which increased the number of circulating haemocytes. Although manganese was shown stressful to the haemocytes and affected their ability to phagocyte, the increased number of haemocytes compensates these impairments. There was seemingly a negative correlation between the accumulation of the metal in the tissues of the animals and their ability to eliminate bacteria. Although Mn does not cause chronic effects on immunity, the expanding areas with bioavailable Mn might have an impact on species composition since some invertebrates become more susceptible to infections.

Keywords: Invertebrates, immune system, haemocytes, manganese (Mn), immunotoxicology, Crustacea, Mollusca, Echinodermata

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